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Bioprocessed Wheat Residues to Recycle Plant Nutrients to the JSC Variable Pressure Growth Chamber during the L/MLSTP Phase III Test

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ABSTRACT

The KSC Breadboard Scale Aerobic Bioreactor (B-SAB) was used to bioprocess inedible wheat crop residues to provide recycled nutrients to support crop growth in the JSC Variable Pressure Growth Chamber (VPGC) as part of the 91 day JSC-Lunar/Mars Life Support Test Project Phase III. To meet the wheat nutrient demand at JSC, the KSC B-SAB was operated at both a higher loading rate (35 gdw L^{-1} compared with 20 gdw L^{-1}) and at a slower retention time (21 days compared with 8 days) than we had used in previous bioreactor (continuous stirred tank reactor - CSTR) studies. The bioreactor operated for 19 weeks—8 weeks startup and steady state stabilization then 11 weeks of operation with the broth harvested weekly. Filtered broth was amended with nutrients and transported to JSC for integration into the VPGC wheat growth component of L/MLSTP Phase III. Biodegradation of JSC wheat residues was a constant 45% during steady state bioreactor operation, and similar to previous B-SAB runs.

INTRODUCTION

Since 1989, the KSC Advanced Life Support Breadboard project has investigated the use of Continuous Stirred Tank Reactors (CSTRs) for bioprocessing solid wastes, namely inedible crop residues [1, 2, 3]. The bioreactors ranged in size from 4 to 120 liter working volumes and were used to conduct studies at both an intermediate-laboratory scale and at the larger breadboard scale. The bioreactors were fully instrumented for computer monitoring and control and were used to process inedible ALS crop residues to accomplish multiple purposes.

Soluble organic compounds are readily leached from crop residues and account for nearly 25% of crop residue carbon [4]. Without biodegradation of these compounds, crop growth on the resulting solution is reduced [5] and growth of microbial biofilms on hardware and root surfaces is prolific and results in biofouling of hydroponic systems. In addition, an increase in denitrification (conversion of desired nitrate to undesired di-nitrogen gas) may occur. Over a wide range of bioreactor retention times (1.3 to 48 days), microbial decomposition consistently reduces the level of soluble organic compounds by 75% [6]. The remaining refractile soluble organics (25%) have little inhibitory effect on crop growth and contributed little to the proliferation of biofilm fouling.

We showed that a CSTR can be used to recycle inorganic nutrients from crop residues back to a hydroponic crop production system. Crop residues were leached in the bioreactor with the soluble minerals recovered in the harvested broth. Again, over a wide range of retention times (1 to 48 days), a significant fraction of the inorganic nutrients contained in crop residues were recovered in bioreactor broth. These broths have been used successfully at both laboratory and breadboard scales to partially replenish crop hydroponic solutions in a number of integrated studies [5]. CSTR bioreactor broths routinely have supplied inorganic nutrients in seed-to-harvest intermediate-scale studies of wheat, potato, and mixed cropping (wheat and potato). At the breadboard scale, two separate studies (wheat and potato) have been conducted. The longest study incorporating bioreactor broth, was a 418-day breadboard production run with 2 levels (5 m^2 crop growing area per level) of white potato.

Two years after the last run of B-SAB, a request was made to demonstrate the biological regeneration of crop nutrients during the Lunar/Mars Life Support Test

Project (L/MLSTP) Phase III at Johnson Space Center (JSC). The overall objective of the Phase III test was to conduct a 91-day regenerative life support system test with four human test subjects demonstrating an integrated biological and physicochemical life support system.

This paper reports the results of this request whereby the KSC B-SAB was used to bioprocess inedible wheat residues with the purpose of producing a hydroponic replenishment solution for use in the JSC Variable Pressure Growth Chamber (VPGC) during the 91 day Phase III test.

MATERIALS AND METHODS

OVERALL PROCESS FLOW - The inedible wheat residues to be bioprocessed at KSC were grown under controlled conditions in the VPGC at JSC [7]. After harvest at JSC, the wheat residues were oven dried at 70°C and reduced in length to approximately 2.5 cm. Upon receipt at KSC, the material particle size was milled to 1 mm diameter with a Wiley mill. This milled wheat residue was stored at 70°C until needed. After aerobic bioprocessing in the KSC Breadboard Scale Aerobic Bioreactor (B-SAB), bioreactor solids (microbial cells and undigested wheat residues) were separated from the soluble, nutrient-laden broth by filtration. The resulting filtrate solution was amended with stock chemicals to bring nutrient levels up to JSC requirements (Table 1), then sent back to JSC where it was used to replenish hydroponic solutions in Side B of the VPGC.

Table 1. JSC requirements for inorganic nutrients in their replenishment solution for hydroponic production of wheat in the VPGC during L/MLSTP Phase III.

Element	JSC requirement, replenishment solution (mmole L^{-1})
N	1500
P	150
K	1350
Ca	150
Mg	196
s	197
Fe	7.96
Mn	0.680
Zn	0.176
Cu	0.095
B	1.74
Mo	0.0018
Na	20

EQUIPMENT AND CONDITIONS - The B-SAB at KSC has a 120 liter working volume. A detailed description of the design and operation of aerobic bioreactors at KSC has been published [1, 2, 3].

Environmental Conditions - B-SAB environmental variables were kept constant. Bioreactor pH was computer controlled at a set point of 6.5 by addition of 1 N nitric acid. Temperature was maintained at 35°C by computer actuation of a solenoid valve that controlled the flow of 45°C water from an external circulating water bath though stainless steel coils wrapped around the bioreactor and insulated. Dissolved oxygen was kept above 2.0 mg L^{-1} by an air flow of 8.0 liters min^{-1} and a stirring rate of 158 rpm.

Bioreactor Process Conditions - Process variables were also controlled. Solids loading was 35 g L^{-1}, which was 1.75 times higher than previous B-SAB runs. This high loading was necessary to meet nutrient concentrations required for the JSC nutrient solution. Milled inedible wheat residues from the JSC-VPGC were introduced into B-SAB by a screw auger located in the bottom of a stainless-steel feed hopper. The auger conveyed solids out of the hopper and into the bioreactor. The volumetric feed rate for dried inedible wheat biomass was kept constant.

The retention time was approximately 21 days and was comparable to other B-SAB runs. A weekly harvest schedule was chosen to match nutrient requirements of the JSC wheat plants. Normally, the amount of oven-dried biomass to be processed in one week (1.4 kg) was loaded into a solids feed hopper, and fed into the B-SAB at a rate of 200 g dry biomass day^{-1}.

Bioreactor inoculum - B-SAB was inoculated with decomposing wheat residues (source, KSC Biomass Production Chamber) from experiments running in one of our intermediate scale aerobic bioreactors. This bioreactor had been in operation for 30 days and was, in turn, inoculated with frozen bioreactor contents from previous studies, in addition to the microflora native to the crop residues and de-ionized water source.

FILTRATION OF BIOREACTOR BROTH - Each week, one-third of the bioreactor contents (40 L) was harvested and immediately filtered to remove solids and to recover soluble nutrients. The bioreactor broth was first passed through a screen (Vibrascreen Corp.) to remove solid particles larger than 43 microns. This resulted in two fractions: a coarse filtered slurry and solids retained by the vibrascreen (mostly undigested plant biomass). In the second stage of the filtering process, the coarse-screened slurry was further filtered through a hollow fiber membrane filtration apparatus to remove particles larger than 0.2 μm. This filtration process produced two fractions: a clear brown liquid (filtrate) devoid of all particles and bacteria greater than 0.2 μm and a thick viscous material (Microfiltration solids -- retentate) that was rich in microbial biomass. Both stage 1 and 2 solids were oven dried and stored for analysis.

NUTRIENT SOLUTION - The filtered bioreactor broth was stored refrigerated at 4°C until shipment to JSC. The concentration of inorganic nutrients in the filtered broth were determined at KSC, using ICP and AA spectrometry methods, prior to making adjustments. Upon receiving the results of these analyses, the broth solution was amended using stock nutrient solutions. The relative concentration of nutrients in the filtered broth was matched to the ratio of macro- and micro-nutrients in JSC stock solutions used for hydroponic production of their wheat.

We desired that 67% of the nutrients for replenishment come from inedible biomass (i.e., given a 33% harvest index for the wheat crop, 67% would be inedible). Nutrient solution amendments were based on ratios derived from potassium levels in KSC filtered broth and requirements for potassium in the JSC stock replenishment formulation. Potassium was selected over other elements because, on a mass basis, it was the largest inorganic component in the filtered broth. Chemical analysis determined the mineral concentrations in the filtered broth and the KSC/JSC amendment ratio (based on K levels) was calculated. Amendment of all elements, not just potassium, were based on this ratio. After initial amendment, a second mineral analysis was done and final mineral additions were made to bring the filtered broth near the desired concentrations. A similar replenishment scheme has been used at KSC for studies that integrate biological processing of crop residues with hydroponic production of crops [4, 5, 8]. The amended solution was stored at 4°C for one week after harvest. After final amendment, the solution was sent by overnight express to JSC.

ANALYTICAL METHODS - Offgas measurements - CO_2 concentration in the B-SAB exit gas (offgas) was measured with an infra-red CO_2 analyzer (LiCor Model LI 6252).

Computer Monitoring - Dissolved oxygen, pH, temperature, gas flow rate, and offgas CO_2 concentration were monitored continuously as described by Finger and Strayer [3]. Hardware for monitoring and control included a SUN Sparc Station and OPTO-22 digital and analog input/output boards. Software (UNDACE V1.9) developed at KSC for monitoring and control of ALS breadboard components was the primary information interface between the operator and the bench-scale bioreactors (6). All monitored parameters were collected at 10 minute intervals and archived on a Hewlett Packard Model 9000 I-50 mainframe computer.

RESULTS AND DISCUSSION

GENERAL BIOREACTOR PERFORMANCE - To meet the nutrient demand of the wheat being grown at JSC, the B-SAB was operated at both a higher loading rate (35 gdw L^{-1} compared with 20 gdw L^{-1}) and at a slower retention time (21 days compared with 8 days) than we had used in previous bioreactor (CSTR) studies. A preliminary bench scale test (Intermediate Scale

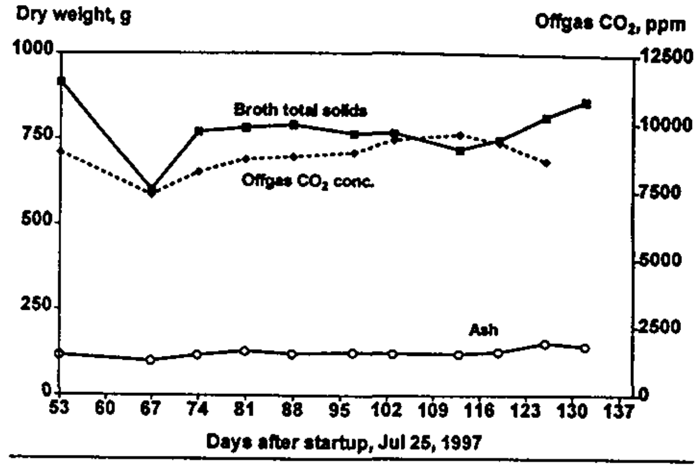

Figure 1 Biological performance of the B-SAB including dry weight of harvested B-SAB total solid components and CO_2 concentration in bioreactor offgas. Total solids are represented by the closed squares and solid line and is the total dry weight (volatile solids plus ash). Concentrations of offgas CO_2 is shown by the solid diamonds with a dashed line.

Aerobic Bioreactor, 8 L working volume) at a high loading rate and 8 day retention time showed minimal problems with CSTR dissolved oxygen concentration and foaming. The two-stage, post-harvest solids separation system – 45 µm vibrascreen + 0.2 µm cross flow filtration—was also tested at this higher solids loading rate.

The bioreactor was started on July 25, 1997, well in advance of the mid-September date when bioreactor broth would be needed for replenishment of VPGC hydroponic nutrient solution. After inoculation, wheat residues were fed into the reactor, without a weekly harvest, for 21 days to allow for a gradual development of an active microbial community. After this startup period, the bioreactor was fed and harvested normally (200 gdw day^{-1}, 1/3 volume harvested weekly) for 32 days to obtain steady state operation and performance.

From day 53 to day 132, bioreactor broth was harvested and filtered weekly, nutrient amendments were made, and the resulting hydroponic replenishment solution sent to JSC. Figure 1 shows the biological performance of the B-SAB during this time. The concentration of total solids in the broth was relatively constant during the middle of the run (days 74 to 118). Average concentration of respired carbon dioxide in the offgas gradually increased during this period.

Due to uncertainty over an assured supply of wheat biomass from the VPGC run around day 60, the bioreactor was not harvested on that day and was not fed for the next week. The decline in bioreactor solids content and in carbon dioxide production reflects this short starvation period and an inadvertent addition of water. Once the decision was made at JSC to continue with the VPGC study, feeding and harvesting of B-SAB recommenced on day 67.

The average degradation of the JSC wheat residues, excluding the starvation period, was 45% of total solids. This rate was considerably better than the best degradation, 37%, observed during the only other run of B-SAB with wheat residues (fed KSC grown wheat, 8 day retention time, pulse fed -- not fed continuously, [3]). The best degradation rate for wheat residues was obtained in a study of retention times using our intermediate scale aerobic bioreactors (1/15 the volume of B-SAB). A volatile solids degradation rate of 55% was obtained during this study at a retention time of 21.7 days.

The mineral output of the bioreactor, as indicated by the dry weight of the ash (Fig. 1) was fairly constant throughout the run. This constancy led to reliability in amending the filtered broth with stock chemicals to bring the composition within JSC requirements.

FILTRATION FRACTIONATION OF BIOREACTOR BROTH. - A diagram of the filtration process is shown in Figure 2, with our nomenclature for each fraction (broth, vibrascreen solids, slurry, microfiltration solids, and filtrate). The average wet weight of the vibrascreen solids was 5.5 kg, approximately 7% dry solids/wet content, and an ash/dry solids content of approximately 5%. The second stage/microfiltration solids had an average wet weight of 3.4 kg, and a dry solids/wet content of approximately 5%, and an ash/dry solids content of approximately 11%. The solids had a consistency similar to pudding.

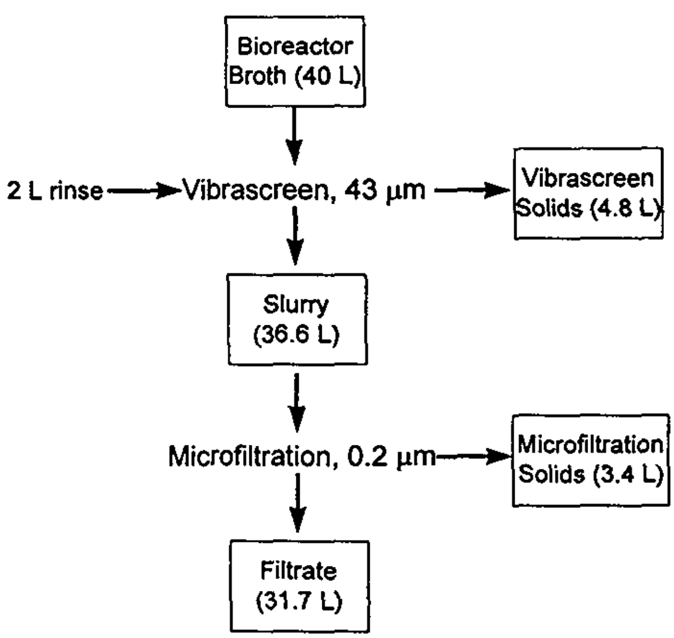

Figure 2. *Schematic diagram of the post-harvest filtration of B-SAB broth.*

Also shown are the measured volumes of liquid fractions, which demonstrate that the solids fractions also contained considerable amounts of liquid. The final filtrate, which was eventually sent to JSC after nutrient amendment, was approximately 75% of the total B-SAB harvest volume. This study was our first attempt to determine the extent of liquid (and thus, nutrient) losses during the separation of bioreactor solids and liquids. Recovery of most of the interstitial liquid from the vibrascreen solids could increase the slurry yield by pressing the solids. The recovery of liquid from the microfiltration solids would be more difficult. The second stage of filtration is more difficult in terms of time and equipment. We recommend that studies be conducted to determine if the microfiltration step is needed. The major issue is the bacterial burden in the slurry and it's effect on crop yield and introduction of undesired bacterial species to the hydroponic system.

The dry weight of various filtration fractions (Figure 3) was also relatively constant after the starvation period, which lasted from day 60 to 67. Of the two vibrascreen fractions, the slurry contained slightly more of the dry weight (average of 53% for all harvests from day 74 to 132) than did the solids. Recovery of dry weight after the vibrascreen averaged 97% of the broth fed into the vibrascreen. For the microfiltration fractions, the dry weight of the filtrate and solids retained by the hollow fiber membranes were equal (average of 50%, each). The dry weight recovery after microfiltration only averaged 81% of the dry weight of the slurry feed (from the vibrascreen).

Ash content of the filtration fractions is a crude indication of the distribution of crop nutrients in the post-processing of B-SAB harvest broth. As shown in Figure

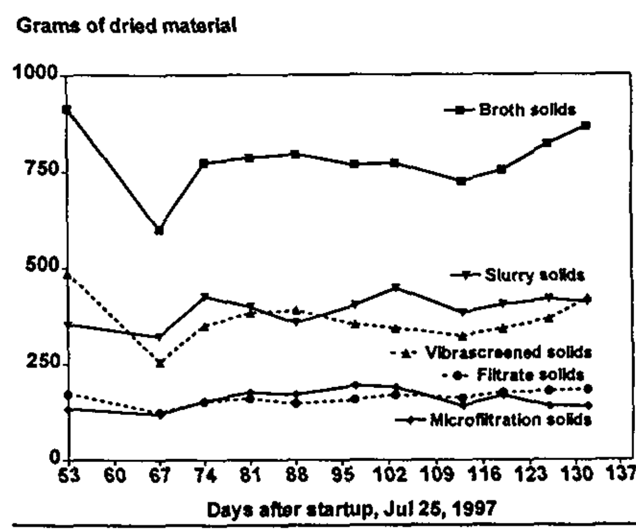

Figure 3. *Timecourse of the distribution of bioreactor broth dry weight in filtration fractions during operation of B-SAB. Lines and symbols are: Broth solids--closed squares with solid line; vibrascreened solids—closed triangles and dashed line; Filtrate solids—closed circles and dashed line; Slurry solids—closed inverted triangle and solid line; and Microfiltration solids; closed diamonds and solid line.*

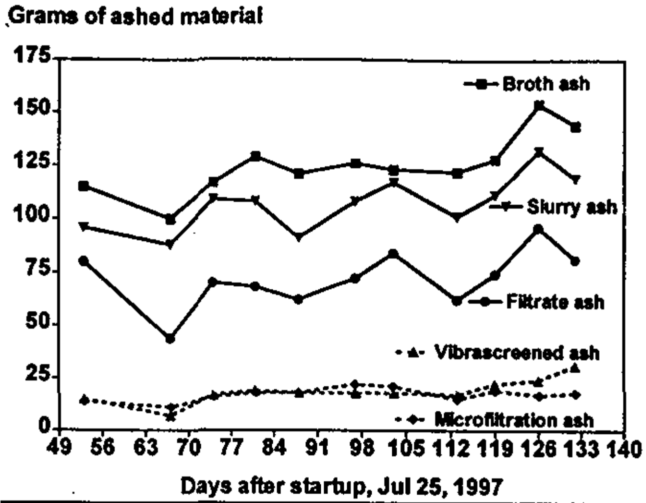

Figure 4. Timecourse of the distribution of bioreactor ash in filtration fractions during operation of B-SAB. Lines and symbols are the same as for Figure 3.

4, the ash was concentrated in the two liquid fractions—slurry and filtrate, as would be desired in a nutrient recovery process for replenishing a crop hydroponic solution. The ash in these liquids represents minerals that were extracted from the wheat residues by physico-chemical processes and by biodegradative processes.

Average ash distribution for days 74 through 132 shows that the vibrascreen filtration stage recovered 100% of the ash in the broth (15% in solids, 85% in slurry). The recovery of ash for the microfiltration stage was 83% of the input slurry ash content with 20% of this in the microfiltration solids and 80% in the filtrate. As shown in Figure 5, recovery of nutrients (i.e., ash) in the filtrate was only 59% of the nutrients that were in the broth. The major "losses" of these nutrients were in the solids fractions, although much of these nutrients were possibly recoverable interstitial liquid. Another major nutrient loss was an unaccounted fraction (difference between broth ash and the sum of filtrate ash, vibrascreen solids ash, and microfiltration solids ash). The hold-up volume contained within the filtration apparatus—hollow-fiber filters, pump, and associated piping—was significant (est. 2 L). In addition to these

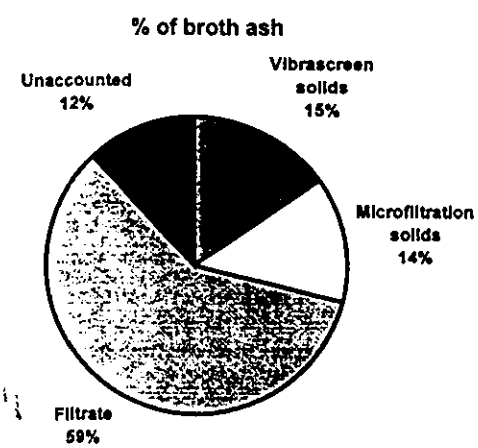

Figure 5. Average percent distribution of bioreactor broth ash in B-SAB post-harvest filtration fractions.

recovery problems, the difference between the slurry ash and the filtrate ash curves in Figure 4 further leads to the conclusion that the elimination of the microfiltration step would give a much better recovery of minerals (as already concluded from the assessment of liquid losses during filtration-Figure 2).

MINERAL CONTENT OF MICROFILTRATION FILTRATE (BEFORE AMENDMENT). - Concentrations of major crop nutrient minerals in the filtered bioreactor broth were relatively constant through day 88 (Figure 6--potassium and nitrogen; Figure 7--calcium, magnesium, and phosphorus). From day 88 to the end of the study, nitrogen and magnesium concentrations remained relatively constant, while calcium fluctuated. Potassium concentration increased by 36% from day 88 to the end of the study while phosphorus showed an increase to day 119 followed by a decrease.

In past studies we have routinely compared mineral concentrations in bioreactor broth with concentrations in an aqueous extraction (leaching at ambient temperature for 2 hr) of the crop residue being fed into the bioreactor [1, 3]. For the current study, only the feed for the last several weeks of the run was leached. Average broth filtrate levels of potassium, calcium, and phosphorus were 102%, 108%, and 105%, respectively of the levels of these nutrients in this leachate, while levels of magnesium were only 63% of the leachate. Total nitrogen levels were considerably higher (157%), but this reflects addition of nitric acid for pH control of the bioreactor. Most of the micro elements were lower in bioreactor filtered broth (Fe—83%, Na—60%, Bo—68%, Cu—40%, and Mn—64%). Only zinc levels were found the be greater (155%) in filtered broth than in leachate. As with all of our previous studies of crop residue leaching and bioprocessing, molybdenum

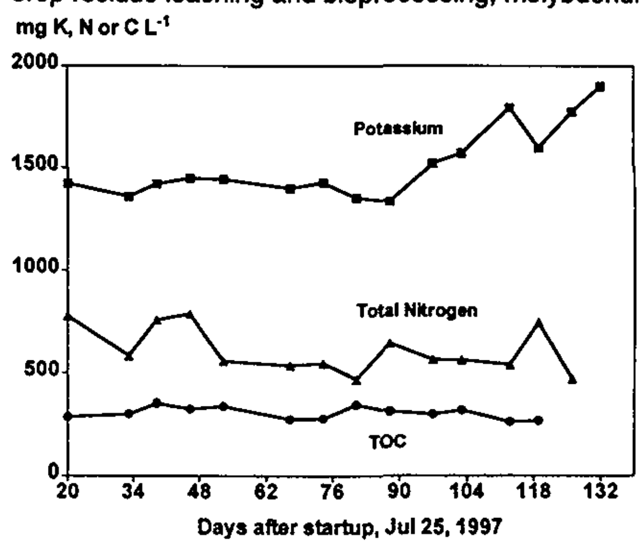

Figure 6. Timecourse of the concentration of potassium, (closed squares) total nitrogen (closed triangles), and soluble total organic carbon (TOC—closed circles) in filtered bioreactor broth (filtrate fraction) prior to adding amendments.

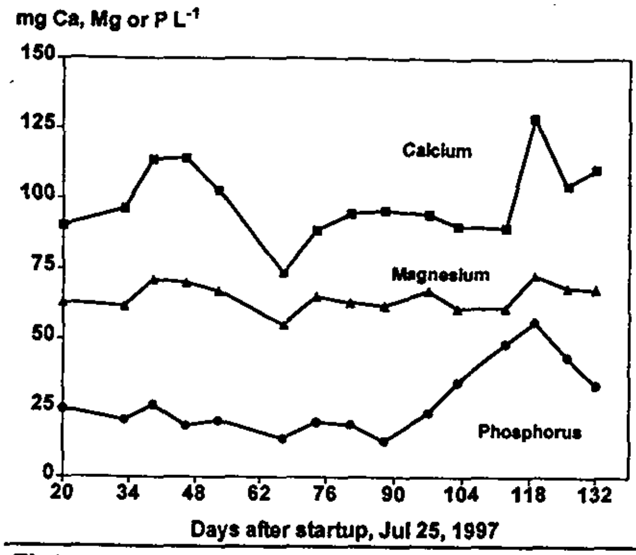

Figure 7. Timecourse of the concentration of calcium, (closed squares), magnesium (closed triangles), and phosphorus (closed circles) in filtered bioreactor broth (filtrate fraction) prior to adding amendments.

was not detected in either leachate or filtered broth. Although aqueous extraction, or leaching, or crop residues is better at recovering microelements, leachates contain significant amounts of soluble organic compounds that lead to problems in hydroponic nutrient delivery systems if not first removed by aerobic biodegradation [4, 9]. These organic compounds can cause increases in biofilms on root and hardware surfaces, root respiration, and denitrification potential in the root zone [10]. Soluble TOC levels in the leachate of JSC-wheat residues ranged between 2100 and 3100 mg C L^{-1}. These levels were reduced to an average of 300 mg C L^{-1} in the B-SAB filtered broth (Figure 6), for an 85% to 90% reduction by bioprocessing. This reduction in soluble TOC is slightly better than the 75% to 80% reduction we have observed in previous B-SAB runs [3].

The filtered broth was amended with stock chemicals to bring levels up to those required by JSC for their nutrient replenishment solution. Figure 8 shows the temporal fluctuations in pre- and post-amendment concentrations for potassium and calcium (as a representative example for the other major elements). Potassium levels are shown because this element was the basis for the amendments. By definition, the filtrate was amended to have "100%" of the potassium required for the JSC replenishment solution. Calcium levels are shown as a representative for the other elements. Overall, after amendment the solution contained 58% of the required concentration of calcium. For other major elements (timecourse data not shown) these percentages were: N, 96%; P, 28%; Mg, 52%; and for the minor elements were: Fe, 22%; Mn 49%; Zn, 171%; Cu, 65%; B, 121%; and Na, 75%.

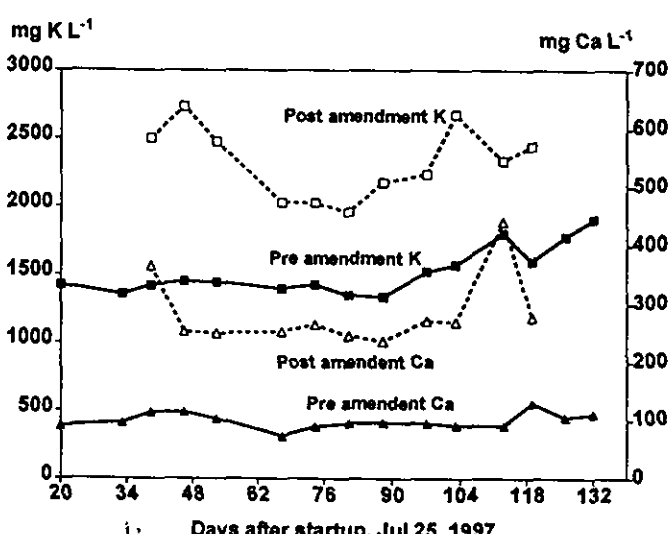

Figure 8. Comparison of pre- and post-amendment levels of potassium (pre- closed squares, post-open squares) and calcium (pre-closed triangles, post- open triangles) of filtered B-SAB broth.

At Johnson Space Center, the amended solutions were introduced into the hydroponic nutrient solution on day 17 of the 91 day Phase III test. Grain yield and total dry biomass production from the wheat plants receiving the amended bioreactor solution was equal or slightly greater than the plants receiving normal nutrient solution prepared from laboratory chemicals [7]. This would indicate a successful utilization of the recycled plant nutrients under conditions of the L/MLSTP Phase III test.

CONCLUSION

A coordinated effort by scientists and engineers at JSC and KSC resulted in the successful biodegradation of L/MLSTP phase III crop residues for the purpose of recycling inorganic nutrients back to the crops. Even though a higher feed rate and slower retention time was used for this study, compared with previous research at KSC, the bioreactor performance was well within the range of past studies. Losses of crop mineral nutrients during bioreactor post-harvest processing—namely water and minerals during microfiltration to remove particulate solids from the desired replenishment solution were noted. We suggest that future studies determine the need for removal of bacterial sized particles and possibly elimination or relacement of the microfiltration step. A separation process that is less prone to holdup losses (e.g., continuous centrifugation) may be utilized.

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